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Germin-like proteins (GLPs) in cereal genomes: gene clustering and dynamic roles in plant defence

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Abstract The recent release of the genome sequences of a number of crop and model plant species has made it possible to define the genome organisation and functional characteristics of specific genes and gene families of agronomic importance. For instance, *Sorghum bicolor*, maize (*Zea mays*) and *Brachypodium distachyon* genome sequences along with the model grass species rice (*Oryza sativa*) enable the comparative analysis of genes involved in plant defence. Germin-like proteins (GLPs) are a small, functionally and taxonomically diverse class of cupin-domain containing proteins that have recently been shown to cluster in an area of rice chromosome 8. The genomic location of this gene cluster overlaps with a disease resistance QTL that provides defence against two rice fungal pathogens (*Magnaporthe oryzae* and *Rhizoctonia solani*). Studies showing the involvement of GLPs in basal host resistance against powdery mildew (*Blumeria graminis* ssp.) have also been reported in barley and wheat. In this mini-review, we compare the close proximity of GLPs in publicly available cereal crop genomes and discuss the contribution that these proteins, and their genome sequence organisation, play in plant defence.

Keywords Germin-like proteins (GLPs) · Disease resistance · Cereal genomes

Introduction

Many genes and gene families in plants have been implicated in specific and quantitative responses to a range of stresses. In times of biotic stress, plants can produce several different types of defence responses and genetic mechanisms for recognising and responding to attack. Qualitative disease resistance to pathogens is mediated by a single resistance (R)-gene whose products can recognise pathogen effectors (Zimmermann et al. 2006; Jones and Dangl 2006). Disease resistance receptor genes (R-genes and host-pattern recognition receptor (HPRR) genes; Kou and Wang 2010) generally make proteins such as receptor-kinases (such as rice *Xa21* (Song et al. 1995) and barley *Rpg1* (Brueggeman et al. 2002)) and nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins. NBS-LRR proteins have highly variable LRR domains which are primarily used for pathogen recognition (Yahiaoui et al. 2004; Caplan et al. 2008; McDowell and Simon 2008; Bhullar et al. 2009; Padmanabhan et al. 2009). Many R-genes have been isolated in crop species using map-based cloning techniques such as *Lr10* (Feuillet et al. 2003), *Lr1* (Cloutier et al. 2007), *Pm3* (Yahiaoui et al. 2004) and *Lr21* (Huang et al. 2003).

Other than receptor genes, defence-responsive or defence-related genes (acting as either activators or suppressors) can respond to pathogen attack through post-translational protein modification and variation of expression (Eulgem 2005; Benschop et al. 2007; Kou and Wang 2010). These race non-specific genes, along with HPRRs in combination, act to provide quantitative disease resistance at a quantitative trait locus (QTL). While possibly performing with multiple

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defensive roles, these genes give an additive affect on the plant leading to a resistant phenotype (Jones and Dangl 2006). This quantitative type of resistance does not give a complete resistant phenotype compared to the single R-gene resistance, or so-called ‘race-specific resistance’ model, but often provides a more durable or broad-spectrum resistance to pathogens (Ayliffe et al. 2008).

Disease resistance QTLs, as well as candidate genes that are known to be involved in disease resistance, have been shown to cluster on particular chromosomal segments in rice (Wisser et al. 2005) and maize (Wisser et al. 2006). The consequence of having multiple genes in a closely linked genome region such as a QTL creates difficulties in studying their effects. However, the availability of whole genome sequences in multiple crop species, along with QTL mapping techniques, has allowed easier identification and analysis of genes linked to quantitative diseases resistance. Studies like those already carried out in rice (Wisser et al. 2006) have looked to fuse QTL and genome data to identify candidate genes for quantitative disease resistance.

Major and minor quantitative disease resistance genes have recently been identified in wheat and rice from a diverse variety of gene families. The wheat fungal resistance QTL, which contains the gene *Lr34* was identified on chromosome 7D and encodes an ATP binding cassette transporter from the pleiotropic drug resistance subfamily (Krattinger et al. 2009). *Lr34* is effective against diseases such as leaf rust, stripe rust and powdery mildew. A stripe rust resistance gene *Yr36*, conferring broad-spectrum resistance at high temperatures was identified on wheat chromosome 6B and encodes a kinase-START protein (Fu et al. 2009). A rice chromosome 4 QTL for the recessive *pi21* gene, conferring durable resistance to blast disease, was identified as a mutated proline-rich protein that contains a

putative heavy metal-binding domain (Fukuoka et al. 2009). Other genes conferring minor disease resistance effects have also been identified in rice (Hu et al. 2008).

In this publication we review germin-like protein (GLP) gene clusters, one of which was recently identified in a study of a diseases resistant QTL on rice chromosome 8 (Manosalva et al. 2008). The germin-like proteins will be reviewed with regards to their physical protein structure, functional contribution to plant defence and taxonomic distribution. We also present Bioinformatics and comparative genomic analysis of cereal plant genomes, that have either been completed or are in draft stages of sequencing or annotation, and propose major germin-like protein clusters that may identify disease resistance loci.

Defining GLPs

Germins and GLPs were originally identified in wheat plants as a specific marker for the start of germination (Lane et al. 1993; Dunwell et al. 2008). These proteins generally code two exons and contain a ‘cupin’ protein domain (PF00190; Finn et al. 2008) at their C terminus (Fig. 1). The cupin protein domain family are a large, functionally diverse super-family (Dunwell and Gane 1998; Dunwell et al. 2000, 2004) that are distantly related to seed storage proteins such as vicilins (Gane et al. 1998). The cupin domain is named for the protein’s classic jellyroll beta-barrel structural domain, with ‘cupa’ meaning small barrel in Latin (Dunwell et al. 2000).

Each member of the protein family contains two amino acid sequence motifs, described as the ‘germin box’ (Lane et al. 1991; Dunwell and Gane 1998; Dunwell 1998; Yamahara et al. 1999). Motif 1-G(x)5HxH(x)3,4E(x)6G and Motif 2-G(x)5PxG(x)

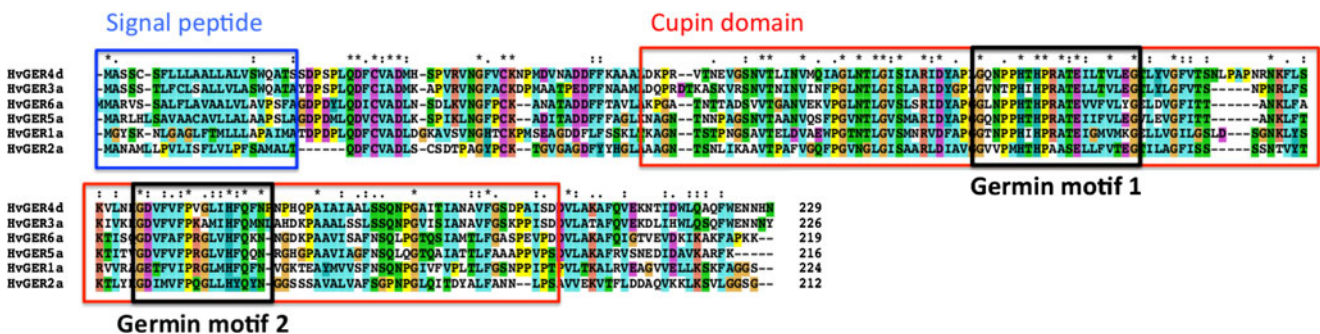


Fig. 1 Multiple sequence alignment of the barley germin amino acid sequences that characterise the GER families in cereals (Druka et al. 2002; Zimmermann et al. 2006; Manosalva et al. 2008). Annotated on the figure is the signal peptide that is found

in most GLPs (blue box), cupin domain (red box) and the two defining germin motifs (black boxes). The asterisks above the sequence indicate amino acid conservation over all six HvGER families

2H(x)3N are both contained in the classic jellyroll beta-barrel structural domain. Crystallographic evidence carried out on a barley germin protein confirmed that six germin proteins (which each bind a single manganese-ion) make up an extremely stable hexamer protein structure (Woo et al. 2000). Each germin protein ‘monomer’ binds to another creating a hexamer structure made from a ‘trimer of dimers’ (Fig. 2). Manganese ions are bound by ligands similar to manganese superoxide dismutase (SOD), with enzyme activity in germin and GLPs confirmed with biochemical evidence (Yamahara et al. 1999; Carter and Thornburg 2000; Woo et al. 2000). Each germin monomer is comprised of an irregular N-terminal extension, the beta-barrel and a C-terminal sequence containing three alpha-helices (Woo et al. 2000). Interestingly the irregular N-terminal domain shape is conserved in many GLPs. In total the hexamer contains about 1,200 amino acids with an approximate molecular mass of 130 kDa (Lane 2002). This is contrasted by the discovery of a single copy GLP from rice that has SOD-activity in its dimeric form (Banerjee and Maiti 2010).

GLPs and other cupin domain-containing proteins are a functionally and taxonomically diverse family of proteins (Table 1). Germins and GLPs are single cupin domain proteins (or monocupins) along with proteins such as microbial phosphonmannose isomerases, AraC-type transcriptional regulators and oxalate oxidase (OXO) enzymes in plants, while bicupins (with two cupin domains) are found in proteins such as seed

storage proteins and oxalate decarboxylases (Khuri et al. 2001). The bicupin globular storage proteins such as legumins (11S) and euvicilins (7S) have also been identified as major food allergens being found in such plants as soybean, peanut, walnut and lentil (Mills et al. 2002). Multiple epitopes have been mapped onto their complex multi-dimer structures, which are often associated with these high thermotolerance proteins (Mills et al. 2002; Xiang et al. 2002; Dunwell et al. 2004). The clustering of proteins in a structure-based phylogeny study of bicupin-containing proteins revealed that N- and C-terminal cupin domains had evolved independently of each other (Agarwal et al. 2009). Sequence-based phylogenetic analyses of all cupin-domain-containing proteins in rice can clearly separate monocupins and bicupins, despite >90% amino acid and nucleotide identity Carrillo et al. (2009).

The high sequence conservation of germins and GLPs over multiple plant species presents difficulties in classification (Dunwell and Gane 1998). Structural characteristics of these proteins offer a more robust system of classification due to the clustering of proteins with conserved functions (Agarwal et al. 2009). The well-conserved homogeneous group of so-called ‘true germins’ are those found to have OXO activity and are almost exclusively found within cereal plant species (Lane et al. 1991; Woo et al. 2000; Bernier and Berna 2001; Carrillo et al. 2009; Davidson et al. 2009). The heterogeneous GLP group of proteins have a far wider taxonomic range in plants and include germin-motif

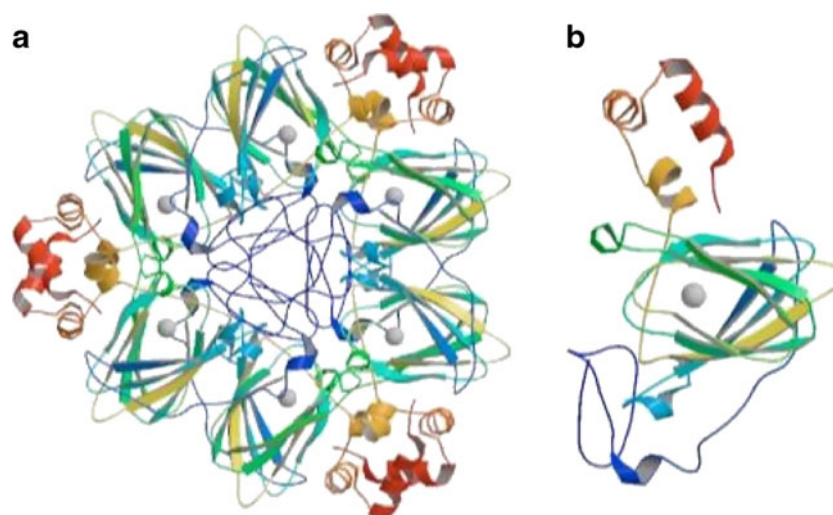


Fig. 2 The hexameric 3D-protein structure of the barley 130 kDa germin protein (**a**) made up of six individual germin monomer proteins (**b**) that each bind one manganese ion each (grey spheres in the middle of **b**). The germin monomer is made up of an alpha-helical C-terminal domain (coloured in red), a barrel structure made up of multiple beta-sheets (green and yellow) and an irreg-

ular N-terminal extension (blue). The protein structure on this barley germin hexamer (PDB Accession: 1FI2) was determined by X-ray crystallisation (Woo et al. 2000) and the figures were taken from the RCSB protein data bank (PDB; Kouranov et al. 2006)

Table 1 Summary of major groups of cupin domain-containing proteins

| Protein | Type | Organisms | Literature cited |
|--|-----------|---------------|---|
| Type II—Phosphomannose isomerases (PMIs) | Monocupin | Microbial | Dunwell and Gane (1998) |
| HTH Transcription factors | Monocupin | Microbial | Aravind and Koonin (1999) |
| AraC-type transcriptional regulators | Monocupin | Microbial | Gallegos et al. (1997); Soisson et al. (1997a, b) |
| Gentisate 1,2-dioxygenases (GDOs) | Monocupin | Microbial | Dunwell et al. (2000) |
| Oxalate decarboxylases | Bicupin | Microbial | Tanner and Bornemann (2000) |
| 7S and 11S seed storage proteins (legumins, globulins, triticin, glutelin and euvicilins) | Bicupin | Plants | Bäumlein et al. (1995); Adachi et al. (2001) |
| Germins | Monocupin | Plants, Fungi | Lane et al. (1993) |
| Germin-like proteins (GLPs) | Monocupin | Plants, Fungi | Dumas et al. (1993) |

containing proteins with an average of 50% amino acid sequence identity that either do not possess OXO activity or have not yet been assigned an enzymatic function (Dumas et al. 1993; Carter et al. 1998; Dunwell et al. 2008).

Multiple studies have examined the role of germins and GLPs in a wide variety of plant systems such as *Arabidopsis* (Membré et al. 1997; Carter et al. 1998), woody azalea (*Rhododendron mucronatum*; Kondo et al. 2008), conifers (Mathieu et al. 2006), soybean (Klink et al. 2007), grapevine (Ficke et al. 2002; Cramer et al. 2007; Godfrey et al. 2007) and Medicago (Doll et al. 2003; Soares et al. 2009). The majority of germin and GLP research has centred on cereal plants, especially rice, barley, maize and wheat, where the protein was first discovered. Six germin subfamilies (GER1-6) were characterised in Druka et al. (2002) with varying enzymatic activities e.g. OXO action in GER1 ('true germin' proteins) and SOD activity in GER2 (Banerjee and Maiti 2010), GER4 and GER5 sub-families. The GER1 subfamily has also been shown to be important for germination and early plant development in plants (Federico et al. 2006). Also, OsGLP1 downregulated transgenic plants in rice (a member of the GER2 subfamily) were shown to induce dwarfism, change cell morphology, cause dramatic increases in sheath blight and blast fungal diseases (Banerjee and Maiti 2010).

Plant defence mechanisms of GLPs

GLPs have been studied in a wide variety of systems and implicated as plant cell defenders in many species, conditions and diseases. These proteins have been shown to be highly resistant to proteases, heat, sodium dodecyl sulphate (SDS) and extreme pH (Lane et al. 1993; Lane 1994; Wei et al. 1998; Carter et al. 1998; Membré et al. 2000; Mahmood et al. 2010). Significant GLPs and/or OXO expression have been shown in environmental conditions such as salt stress (Hurkman

et al. 1991, 1994; Cramer et al. 2007), aluminium stress (Houde and Diallo 2008) and drought stress (Ke et al. 2009). These genes are also expressed when attacked by fungal pathogens (Schweizer et al. 1999; Donaldson et al. 2001; Liang et al. 2001; Ficke et al. 2002; Zimmermann et al. 2006; Manosalva et al. 2008), bacteria and viruses (Park et al. 2003) and Erysiphe nectar infections (Godfrey et al. 2007). They are expressed in a diverse range of tissues and can act against parasites like nematodes (Knecht et al. 2010) and insects (Ramputh et al. 2002; Lou and Baldwin 2006).

So far germins and GLPs have been shown to function by using three main enzyme actions. Studies originally from barley revealed that germins have an OXO enzyme action (Lane et al. 1993; Dumas et al. 1993). OXOs act by catalysing the production of CO_2 and H_2O_2 (Requena and Bornemann 1999), the latter being present and implicated in plant defence (Wojtaszek 1997a, b). As mentioned above, SOD activity has been identified in previous studies on germins (Woo et al. 2000) and GLPs (Yamahara et al. 1999) and they work through the conversion of toxic reactive oxygen species (ROS) to H_2O_2 (Kukavica et al. 2005). Additionally, a barley GLP was identified to have a different ADP glucose pyrophosphatase/phosphodiesterase (AGPPase) enzyme activity (Bernier and Berna 2001; Mahmood et al. 2010), which may help to control metabolic flow towards starch, cell wall polysaccharides, glycoproteins and glycolipids in plants (Rodríguez-López et al. 2001).

A previous study into germin isoforms (Lane et al. 1992) identified that approximately 40% of germins in wheat embryonic tissue are cell wall-associated and important for plant development. To date, all GLPs analysed possess N-terminal secretory signals, which further indicates cell wall/extracellular matrix targeting (Lane et al. 1992; Heintzen et al. 1994; Lane 1994; Bernier and Berna 2001; Zimmermann et al. 2006). Other cell wall localisation studies have also confirmed this cell surface association (Heintzen et al. 1994; Davidson et al. 2010). A cell-wall-associated GLP in

rice was also shown to be located in higher abundance in sub-epidermal cells (Banerjee and Maiti 2010). It is believed that GLPs might also possess a structural role in cross-linking the cell wall after a pathogen attack (Christensen et al. 2004).

Most studies investigating the physiological cell defence mechanisms of GLPs have focused on the expression and defence mechanisms of GLPs in the apoplast (Felle et al. 2005), with their cell-wall interaction enabling early response to pathogens (Davidson et al. 2010). GLPs have been shown to increase in expression within plant cells after infection (causing the production of ROS such as H_2O_2) and form an ‘oxidative burst’ response (Wojtaszek 1997a; Averyanov 2009). H_2O_2 is also involved in cell signalling in the apoplast. A transgenic study in sugar beet indicated that expression of BvGLP-1 constitutively activates the expression of other defence-related proteins and downregulates others (Knecht et al. 2010). Transgenic ectopic expression studies in Soybean (Donaldson et al. 2001) and gene silencing in epidermal cells of *Nicotiana attenuata* (Lou and Baldwin 2006) also indicated the expression of GLP-encoding genes in cell wall regions proximal to the pathogen attack or wounding site.

GLP gene clusters in currently available cereal genome sequences

Two studies of GLP expression in barley (Zimmermann et al. 2006) and a QTL on chromosome 8 of rice (Manosalva et al. 2008) have shown the complex nature of GLP genes in cereal genomes and confirmed their involvement in broad-level disease resistance. Both studies analysed plant interaction with fungal pathogens such as powdery mildew (*Blumeria graminis* f.sp. *hordei*) in barley and rice blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctania solani*) diseases in rice. Pre-genome sequence analyses carried out on each rice chromosome in Wisser et al. (2005) also indicated that the chromosome 8 disease resistance QTL was indeed a ‘hotspot’ for different disease resistance genes. Developmental transcript analysis of barley tissues showed a broad range of expression of five germin sub-families previously outlined in Druka et al. (2002). Gene silencing through RNA interference showed that transient silencing of GER3 and GER5 sub-families in barley and transient over-expression of GER4 and GER5 protected epidermal cells from powdery mildew attack. The silencing of GER4 led to high susceptibility to pathogen attack (Zimmermann et al. 2006).

Similar results were found in rice (Manosalva et al. 2008) except a fully sequenced rice genome enabled the authors to identify genes involved in a rice blast disease associated QTL. Eight GLPs were identified to form the QTL on chromosome 8 in a tightly linked cluster and after RNA silencing of each GLP gene in the cluster, the susceptibility to the two rice diseases mentioned above increased. Like in Zimmermann et al. (2006), the GER4 subfamily was identified to contribute most to diseases resistance.

The recent studies identifying GLP gene clusters in both the rice chromosome 8 QTL region studied in Manosalva et al. (2008) and a sequenced region in barley (Himmelbach et al. 2010) indicated a strong connection between both GLP clusters and disease resistance phenotypes. To identify any more GLP clusters the publicly accessible genome sequences of *Brachypodium distachyon*, rice (*Oryza sativa*), *Sorghum bicolor* and maize (*Zea mays*) were assayed to identify GLP gene locations. One rice GLP (OsGLP8-1) from the chr8 QTL region (Manosalva et al. 2008) was used to query the JGI Phytozome comparative genomics resource (<http://www.phytozome.net/>). The OsGLP8-1 belonged to the ‘hypothetical grass post-duplication gene’ family (Cluster 22740912), which encompasses all the rice chromosome 8 GLPs from the QTL region defined in (Manosalva et al. 2008).

The chromosomal locations of all genes within the family were identified and defined as a cluster if three or more GLPs were found in close proximity (a region with less than 100 kb between each gene member). Genes were renamed according to their position on each chromosome, mirroring the nomenclature used to describe the rice GLP genes in Manosalva et al. (2008) e.g. OsGLP8-1 is the first gene family member on rice chromosome 8 (Table 2). One *Sorghum bicolor* GLP did not have a designated chromosome and was therefore named SbGLP-unk. All genes contained within the gene family contained a cupin domain (Pfam domains PF07883 and PF00190) except for BdGLP3-7 which contains a F-BOX domain (PF00646). The size of BdGLP3-7 is almost double the size of all other genes in the family and it is likely that this is due to an insertion within the cupin domain. Five GLPs found on chromosome 10 in maize were not classified as a cluster (ZmGLP10-1 to ZmGLP10-5) as they were spaced over 800 kb. The size and number of GLPs within each cluster are detailed in Table 3.

Six clusters were identified in the four genomes and contained 56 out of the 65 genes (86%) found within the gene family, indicating that GLPs within this family are likely to be contained in clusters. Syntenic genome segment analysis from whole genome analyses (Salse

Table 2 All genes found in the hypothetical gene (#22740912) family (Phytozome 5 families; <http://www.phytozome.net/>) that were used to query GLP clustering in completely sequenced post-duplication grass genomes

| Genome | Chromosome | Chromosome position | Cluster name | Annotated name ^a |
|--------------------------------|------------|---------------------|--------------|-----------------------------|
| <i>Brachypodium distachyon</i> | 2 | 18393515–18394672 | BdGLP2-1 | Bradi2g21010.1 |
| | 3 | 13504258–13505260 | BdGLP3-1 | Bradi3g15190.1 |
| | | 13507473–13508475 | BdGLP3-2 | Bradi3g15200.1 |
| | | 13511002–13512125 | BdGLP3-3 | Bradi3g15210.1 |
| | | 13513339–13514481 | BdGLP3-4 | Bradi3g15220.1 |
| | | 13525866–13526904 | BdGLP3-5 | Bradi3g15240.1 |
| | | 15477075–15477893 | BdGLP3-6 | Bradi3g17310.1 |
| | | 15480755–15484032 | BdGLP3-7 | Bradi3g17320.1 |
| <i>Oryza sativa</i> | 8 | 15517276–15518266 | BdGLP3-8 | Bradi3g17330.1 |
| | | 5185878–5186701 | OsGLP8-1 | LOC_Os08g08920.1 |
| | | 5207388–5208572 | OsGLP8-2 | LOC_Os08g08960.1 |
| | | 5221217–5222314 | OsGLP8-3 | LOC_Os08g08970.1 |
| | | 5227825–5229037 | OsGLP8-4 | LOC_Os08g08980.1 |
| | | 5232771–5233801 | OsGLP8-5 | LOC_Os08g08990.1 |
| | | 5238002–5239151 | OsGLP8-6 | LOC_Os08g09000.1 |
| | | 5241498–5242660 | OsGLP8-7 | LOC_Os08g09010.1 |
| | 12 | 5247669–5248835 | OsGLP8-8 | LOC_Os08g09020.1 |
| | | 5253289–5254233 | OsGLP8-9 | LOC_Os08g09040.1 |
| | | 5259155–5260302 | OsGLP8-10 | LOC_Os08g09060.1 |
| | | 5263250–5264392 | OsGLP8-11 | LOC_Os08g09080.1 |
| | | 7993396–7994721 | OsGLP8-12 | LOC_Os08g13440.1 |
| | | 2687758–2688828 | OsGLP12-1 | LOC_Os12g05840.1 |
| | | 2691487–2692513 | OsGLP12-2 | LOC_Os12g05860.1 |
| | | 2695409–2696216 | OsGLP12-3 | LOC_Os12g05870.1 |
| | | 2698177–2699274 | OsGLP12-4 | LOC_Os12g05880.1 |
| | 6 | 2974644–2975922 | SbGLP6-1 | Sb06g001690.1 |
| | | 7102087–7103153 | SbGLP7-1 | Sb07g005240.1 |
| | | 7160130–7161537 | SbGLP7-2 | Sb07g005250.1 |
| | | 7239661–7240653 | SbGLP7-3 | Sb07g005260.1 |
| | | 7251112–7252308 | SbGLP7-4 | Sb07g005270.1 |
| | | 7259660–7260808 | SbGLP7-5 | Sb07g005280.1 |
| | | 7306801–7307936 | SbGLP7-6 | Sb07g005290.1 |
| | | 7315270–7316197 | SbGLP7-7 | Sb07g005300.1 |
| | | 7409465–7410307 | SbGLP7-8 | Sb07g005310.1 |
| | | 7419332–7420175 | SbGLP7-9 | Sb07g005320.1 |
| | | 7426073–7426916 | SbGLP7-10 | Sb07g005330.1 |
| | | 7435638–7436234 | SbGLP7-11 | Sb07g005340.1 |
| <i>Sorghum bicolor</i> | 7 | 7453736–7454803 | SbGLP7-12 | Sb07g005350.1 |
| | ? | ? | SbGLP-unk | Sb0737s002010.1 |
| | | | | |
| | 2 | 102590903–102592174 | ZmGLP2-1 | GRMZM2G045809_P01 |
| | 3 | 28196791–28197411 | ZmGLP3-1 | AC190772.4_FGP011 |
| | | 28210385–28211585 | ZmGLP3-2 | GRMZM2G030772_P02 |
| | | 28242531–28243821 | ZmGLP3-3 | GRMZM2G149714_P01 |
| | | 28261457–28262833 | ZmGLP3-4 | GRMZM2G093622_P01 |
| | | 28271569–28273041 | ZmGLP3-5 | GRMZM2G093606_P01 |
| | | 28304728–28305909 | ZmGLP3-6 | GRMZM2G093554_P01 |
| | | 28326084–28327223 | ZmGLP3-7 | GRMZM2G157364_P01 |
| | | 28331328–28332493 | ZmGLP3-8 | GRMZM2G157298_P01 |
| | | 28354736–28355846 | ZmGLP3-9 | GRMZM2G148026_P01 |
| | | 28361093–28362101 | ZmGLP3-10 | GRMZM2G148014_P01 |
| | | 28410680–28411226 | ZmGLP3-11 | GRMZM2G155506_P01 |
| | | 28468821–28470086 | ZmGLP3-12 | GRMZM2G165839_P01 |
| | | 28476442–28477847 | ZmGLP3-13 | GRMZM2G074443_P01 |
| | | 28486541–28487446 | ZmGLP3-14 | GRMZM2G105940_P01 |
| | | 28491220–28492488 | ZmGLP3-15 | GRMZM2G093076_P01 |
| | | 28515885–28517050 | ZmGLP3-16 | GRMZM2G072965_P01 |
| | | 28560860–28561920 | ZmGLP3-17 | GRMZM2G176798_P01 |
| <i>Zea mays</i> | | 28566314–28576866 | ZmGLP3-18 | GRMZM2G471355_P01 |

Table 2 (continued)

| | Genome | Chromosome | Chromosome position | Cluster name | Annotated name ^a |
|--|--------|------------|---------------------|--------------|-----------------------------|
| ^a Annotated name in respective genome annotation databases contained within the http://www.phytozome.net comparative genomics site | | | 28591509–28592973 | ZmGLP3-19 | GRMZM2G170829_P01 |
| | | | 28598452–28599606 | ZmGLP3-20 | GRMZM2G170857_P01 |
| | | 10 | 42520607–42521751 | ZmGLP10-1 | GRMZM2G178817_P01 |
| | | | 42648230–42649497 | ZmGLP10-2 | GRMZM2G071390_P01 |
| | | | 43077044–43078521 | ZmGLP10-3 | GRMZM2G049930_P01 |
| | | | 43234112–43235435 | ZmGLP10-4 | GRMZM2G012530_P01 |
| | | | 43263304–43264488 | ZmGLP10-5 | GRMZM2G087111_P01 |

et al. 2008; Schnable et al. 2009; Initiative et al. 2010) indicate that five out of the six clusters are located on an ancestral genome segment, the same segment that contains the chromosome 8 GLP cluster in rice (Manosalva et al. 2008).

All of the 65 genes involved in the above analyses belong to the GER3 and GER4 germin subfamilies as characterised by previous studies in barley (Druka et al. 2002; Zimmermann et al. 2006). As shown on Fig. 3, genes from a species-specific cluster were usually grouped together indicating that one or more genes were duplicated multiple times at that region. Duplication of the GER4 sub-family members in the chromosome 8 QTL genes (OsGLP8-1 to OsGLP8-12) was confirmed in Davidson et al. (2010) based on their proximity to each other and similarities within the sequence and putative regulatory sequences. However, genes from a chromosomal gene cluster in the same species were not exclusively from the same GER sub-family (e.g. out of the 11 genes in the rice chromosome 8 cluster, five were grouped with the GER3 subfamily and seven grouped with GER4). This suggests diversification of duplicated genes within the loci, as was also suggested in Manosalva et al. (2008).

The expressed GER3 and GER4 subfamilies of GLPs contributed the most to resistance against pow-

dery mildew in wheat and barley (Christensen et al. 2004; Zimmermann et al. 2006) and both powdery mildew and blast fungus in rice (Manosalva et al. 2008). The cluster of eight functional GLP genes on barley chromosome 4H were all identified to be from the GER4 sub-family (Himmelbach et al. 2010). This barley GLP cluster did not show synteny to the other GLP clusters in *Sorghum*, *Brachypodium*, rice and maize, however its presence on an ancestral genome segment could not be verified (Salse et al. 2008).

The high sequence similarity between family members in close proximity suggests functional redundancy of GLP proteins (Zimmermann et al. 2006). This is one possible explanation for the clustering of GLPs in cereal genomes. It was suggested in Manosalva et al. (2008) that as GLP genes are amplified through duplication, the protein family diversifies (Kafri et al. 2006) and evolves new functional or structural roles. Agarwal et al. (2009) suggested that the functional diversity of cupin domain-containing proteins is believed to be due to variations in the length of α -strands and greater conformation freedom in folds holding metal binding residues.

The acquisition of new functional roles through duplication was highlighted in rice where the chromosome 8 GLP cluster contributed far more to resistance

Table 3 Clustering of the genes in the ‘hypothetical grass post-duplication gene’ family (Cluster 22740912) from the Joint Genome Institute (JGI)/Center for Integrative Genomics (CIG) comparative genomics website (<http://www.phytozome.net>)

| Species | Genes | Number of gene clusters | GLP clusters | | |
|--------------------------------|-------|-------------------------|--------------|-----------------|--------------------------|
| | | | Chromosome | Number of genes | Approximate cluster size |
| <i>Brachypodium distachyon</i> | 9 | 3 | Chr 3 | 5 | 21 kb |
| | | | Chr 3 | 3 | 41 kb ^a |
| <i>Oryza sativa</i> | 16 | 2 | Chr 8 | 12 | 80 kb^b |
| | | | Chr 12 | 4 | 12 kb |
| <i>Sorghum bicolor</i> | 14 | 2 | Chr 7 | 12 | 353 kb |
| <i>Zea mays</i> | 26 | 3 | Chr 4 | 20 | 405 kb |
| | 65 | 10 | | 52 | |

The bold type chromosome of the major germin clusters indicate the clusters found on syntenic genome segments between the cereal species (i.e. **Bd3/Os8/Sb7/Zm4**)

^aThere are two GLP clusters in close proximity on Bd3

^bEleven of the 12 rice chr8 genes are found in a 80 kb cluster, however found the gene OsGLP8-12 (located over 2 Mb away) to be within the QTL boundary

Fig. 3 Maximum Parsimony phylogenetic tree (100 bootstraps with sequence order re-arranging over 100 data sets) of the genes in the ‘hypothetical grass post-duplication gene’ family (Cluster 22740912) from the Joint Genome Institute (JGI)/Center for Integrative Genomics (CIG) comparative genomics website (<http://www.phytozome.net>). The six barley germin genes (HvGER1a, HvGER2a, HvGER3a, HvGER4d, HvGER5a and HvGER6a) that characterise the subfamily classification (Druka et al. 2002; Zimmermann et al. 2006; Manosalva et al. 2008) are boxed and a GLP from *Arabidopsis thaliana* (At1g02335) were used as comparison (boxed sequences). All other genes contained within the family were coloured according to their species with *Sorghum* in green, *Brachypodium* in orange, rice in blue and maize in red. Low bootstrap values are coloured red on the tree branches

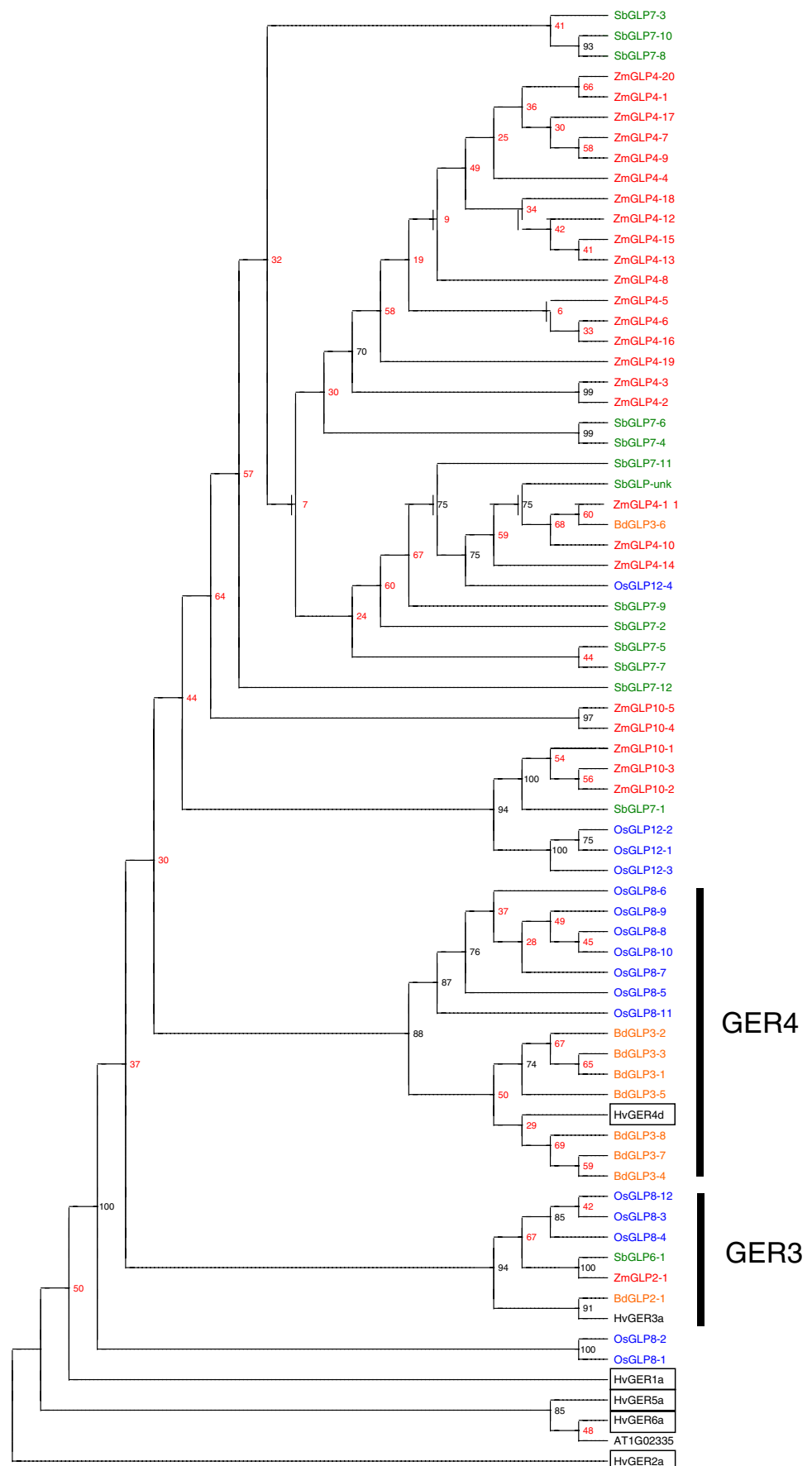
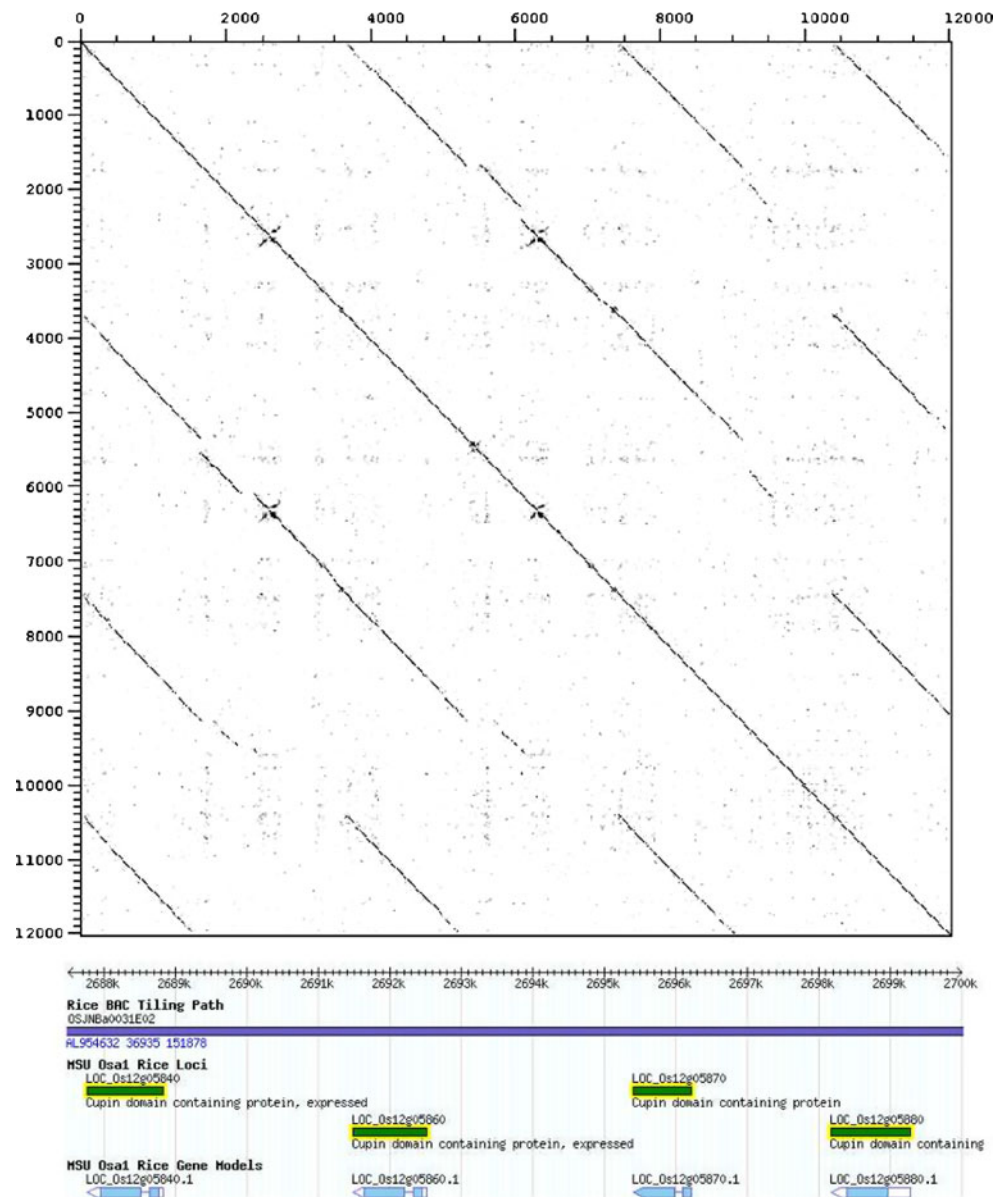


Fig. 4 A self dot-plot of a 12 kb sequence on rice chromosome 12 (2,687,500–2,699,500 bp) that contains four GLP genes. Located below the figure is the corresponding sequence annotation from the MSU (formerly TIGR) rice genome annotation database (Ouyang et al. 2007). The four GLP genes are coloured in green and highlighted in yellow and the red bars indicate repeats from the Oryza repeat database (Ouyang and Buell 2004)



than the smaller chromosome 12 cluster that contained only four genes (Manosalva et al. 2008). This four-gene cluster over 12 kb shows a duplicated nucleotide sequence pattern (Fig. 4), with the duplications likely to be recent and less likely to have evolved new functional attributes. Tandem duplication of genes from the closely related OXO cupin subclasses has also been reported (Carrillo et al. 2009).

The most recent GLP cluster identified in barley defined an interesting cyclic gene ‘birth and death’ scenario for the GLP genes within a cluster (Himmelbach et al. 2010) instead of a typical ‘pseudogenisation’ affect on multiple gene duplicates at a single loci (Ohno 1970). Mutations within regulatory elements showed to

enhance transcript dosage and functional redundancy by pathogen-induced promoter activity. Conserved regulatory elements in GLP promoter sequences have also been shown to be responsive to environmental stresses and growth factors (Mahmood et al. 2010). The evolutionary driving force behind localised gene expansion, suggested in Himmelbach et al. (2010), was the enhancement of transcript dosage encoding functionally redundant proteins in a robust manner rather than the diversification of local genes paralogs.

The syntenic GLP cluster region on maize chromosome 4 also contains multiple QTLs for diseases like ear and stalk rot, common smut, common rust, southern rust, gray leaf spot and northern corn leaf

blight (Wisser et al. 2006). Rice GLPs and OXO gene clusters were also shown to co-localise with multiple published QTLs, with the exception of the four-gene chromosome 12 gene cluster (Davidson et al. 2009). These regions outlined in Davidson et al. (2009) are associated with phenotypes such as bacterial blight, brown plant hopper, leaf and neck blast, yellow mottle virus, sheath rot and sheath blight.

GLP/OXO gene clusters were identified to be located in the boundaries of multiple QTLs on chromosome 8 (Manosalva et al. 2008) and chromosome 3 (Liu et al. 2009; Ramalingam et al. 2003; Sirithunya et al. 2002; Wu et al. 2004; Zou et al. 2000; Tabien et al. 2002; Pinson et al. 2005; Chen et al. 2003). The rice chromosome 3 multiple QTL region is syntenic to maize chromosomes 1 (distal region of both arms), 5 (distal short arm) and 9 (distal long arm; from Salse et al. (2008), all of which contain multiple published QTLs (Wisser et al. 2006). These regions, along with regions where single GLP/OXO genes are located in multiple QTL regions, are good candidates for future disease resistance (Davidson et al. 2009). And despite large amounts of segmental genome variation through duplications, chromosome fusions, and translocations since originating from their ancestral genome 90 MYA, there is a high degree of synteny between ancestral genome segments in cereal genomes (Salse et al. 2008). So by combining metagenome analysis with GLP cluster information, research from model organisms such as rice and maize may be transferred to organisms whose genomes are historically difficult and complex to sequence (Gill et al. 2004; Schulte et al. 2009).

Wheat and barley are two such genomes which are difficult to sequence due to a high proportion of repetitive DNA. GLPs contained within the GLP clusters located on Sorghum chromosome 7 and maize chromosome 4 are all separated by large stretches of repetitive DNA, most notably LTR Retrotransposons, which increase the length (and numbers of genes) of the GLP clusters in both species (355 kb in Sorghum and 405kb in maize; Table 2) compared to the syntenic rice and Brachypodium clusters. Transposable elements (TEs), found in high copy numbers within plant genomes such as wheat, maize and barley, may also be responsible for duplications of GLPs. TE-mediated gene sequence movement has been shown in maize by CACTA DNA Transposons (Li et al. 2009) and *Helitron* elements (Lai et al. 2005). Localised gene duplications of GLPs in TE-rich sequence has also been identified on a disease resistance loci in wheat (J. Breen and R. Mago, unpublished data).

The presence of TEs and smRNAs around GLP clusters may also affect the regulation of each gene within

the GLP cluster. It would be speculative to suggest that an epigenetic affect, such as the affect of tandem repeats at the paramutation loci in maize (Chandler and Alleman 2008), may play a role in localised gene duplications of GLPs. Such a mechanism may account for the clustering of GLPs in cereals and the affect of QTLs to provide durable plant resistance to pathogen attack. Small RNA regulation has already been shown to play important roles in disease resistance gene clusters in *Arabidopsis thaliana* (Yi and Richards 2007). Davidson et al. (2009) scanned small RNA libraries and found instances of regulation of duplicated GLP genes. Within the GLP cluster at the QTL region on chromosome 8 (Manosalva et al. 2008), an insertion within the promoter sequence of OsGLP8-6 (a GER4 family member) enhanced expression in two resistant rice (ssp. indica) cultivars, while higher expression of OsGLP8-12 (a GER3 family member) was identified in susceptible cultivars (Davidson et al. 2010). The authors in Davidson et al. (2010), along with small RNA data in Davidson et al. (2009), suggest regulation through both small RNAs and regulatory domain variation are responsible for the variation of expression in the important GER3 and GER4 subfamilies in certain tissues.

Conclusion

Studies into QTLs conferring durable and/or broad-spectrum disease resistance phenotypes in cereals are an important step in crop improvement. A recent publication identifying a genomic GLP cluster linked to a disease resistance QTL in rice, along with other studies identifying their defensive actions, have highlighted the importance of this gene family. The large increase in sequence data from plant genomic sequencing projects, especially important cereal crops, has allowed researchers to identify candidate disease resistance hotspots and merge with current QTL mapping data.

In this mini-review we consider that GLP genes are involved in plant defence and reviewed recent literature that discusses possible mechanisms of non-race specific disease resistance. GLP clusters (three or more GLPs found in close proximity to each other) are common in cereal genome sequences and they could provide new opportunities to increase non-race specific disease resistance in crop species. The four major syntenic GLP clusters in maize, *Sorghum*, *Brachypodium* and rice are also interesting models in comparing how gene loci evolve and respond to pathogens. The interplay between localised gene duplications, small RNAs,

TEs and regulatory elements in promoter sequences could interact to affect GLP expression and its ability to defend plants cells using a broad-level and durable resistance mechanism.

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References

- Adachi M, Takenaka Y, Gidamis AB, Mikami B, Utsumi S (2001) Crystal structure of soybean proglycinin a1ab1b homotrimer. *J Mol Biol* 305(2):291–305
- Agarwal G, Rajavel M, Gopal B, Srinivasan N (2009) Structure-based phylogeny as a diagnostic for functional characterization of proteins with a cupin fold. *PLoS ONE* 4(5):e5736
- Aravind L, Koonin EV (1999) Dna-binding proteins and evolution of transcription regulation in the archaea. *Nucleic Acids Res* 27(23):4658–4670
- Averyanov A (2009) Oxidative burst and plant disease resistance. *Front Biosci (Elite Ed)* 1:142–152
- Ayliffe M, Singh R, Lagudah E (2008) Durable resistance to wheat stem rust needed. *Curr Opin Plant Biol* 11(2):187–192
- Banerjee J, Maiti MK (2010) Functional role of rice germin-like protein1 in regulation of plant height and disease resistance. *Biochem Biophys Res Commun* 394(1):178–183
- Bäumlein H, Braun H, Kakhovskaya IA, Shutov AD (1995) Seed storage proteins of spermatophytes share a common ancestor with desiccation proteins of fungi. *J Mol Biol* 41(6):1070–1075
- Benschop JJ, Mohammed S, O'Flaherty M, Heck AJR, Slijper M, Menke FLH (2007) Quantitative phosphoproteomics of early elicitor signaling in arabidopsis. *Mol Cell Proteomics* 6(7):1198–1214
- Bernier F, Berna A (2001) Germins and germin-like proteins: plant do-all proteins. But what do they do exactly? *Plant Physiol Biochem* 39:545–554
- Bhullar NK, Street K, Mackay M, Yahiaoui N, Keller B (2009) Unlocking wheat genetic resources for the molecular identification of previously undescribed functional alleles at the pm3 resistance locus. *Proc Natl Acad Sci USA* 106(23):9519–9524
- Brueggeman R, Rostoks N, Kudrna D, Kilian A, Han F, Chen J, Druka A, Steffenson B, Kleinohs A (2002) The barley stem rust-resistance gene *rpg1* is a novel disease-resistance gene with homology to receptor kinases. *Proc Natl Acad Sci USA* 99(14):9328–9333
- Caplan J, Padmanabhan M, Dinesh-Kumar SP (2008) Plant nb-*lrr* immune receptors: from recognition to transcriptional reprogramming. *Cell Host Microbe* 3(3):126–135
- Carrillo M, Goodwin P, Leach J, Leung H, Cruz CV (2009) Phylogenomic relationships of rice oxalate oxidases to the cupin superfamily and their association with disease resistance qtl. *Rice* 2(1):67–79
- Carter C, Thornburg RW (2000) Tobacco nectarin i. Purification and characterization as a germin-like, manganese superoxide dismutase implicated in the defense of floral reproductive tissues. *J Biol Chem* 275(47):36726–36733
- Carter C, Graham RA, Thornburg RW (1998) *Arabidopsis thaliana* contains a large family of germin-like proteins: characterization of cDNA and genomic sequences encoding 12 unique family members. *Plant Mol Biol* 38(6):929–943
- Chandler V, Alleman M (2008) Paramutation: epigenetic instructions passed across generations. *Genetics* 178(4):1839–1844
- Chen H, Wang S, Xing Y, Xu C, Hayes PM, Zhang Q (2003) Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *pyricularia grisea* in rice and barley. *Proc Natl Acad Sci USA* 100(5):2544–2549
- Christensen AB, Thordal-Christensen H, Zimmermann G, Gjetting T, Lyngkjaer MF, Dudler R, Schweizer P (2004) The germinlike protein *glp4* exhibits superoxide dismutase activity and is an important component of quantitative resistance in wheat and barley. *Mol Plant-Microb Interact* 17(1):109–117
- Cloutier S, McCallum BD, Loutre C, Banks TW, Wicker T, Feuillet C, Keller B, Jordan MC (2007) Leaf rust resistance gene *Lr1*, isolated from bread wheat (*triticum aestivum* L.) is a member of the large *psr567* gene family. *Plant Mol Biol* 65(1–2):93–106
- Cramer GR, Ergül A, Grimplet J, Tillett RL, Tattersall EAR, Bohlman MC, Vincent D, Sonderegger J, Evans J, Osborne C, Quilici D, Schlauch KA, Schooley DA, Cushman JC (2007) Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Funct Integr Genomics* 7(2):111–134
- Davidson R, Reeves P, Manosalva P, Leach J (2009) Germins: a diverse protein family important for crop improvement. *Plant Sci* 3(1):43–55
- Davidson RM, Manosalva PM, Snelling J, Bruce M, Leung H, Leach JE (2010) Rice germin-like proteins: Allelic diversity and relationships to early stress responses. *Rice* 3:43–55
- Doll J, Hause B, Demchenko K, Pawlowski K, Krajinski F (2003) A member of the germin-like protein family is a highly conserved mycorrhiza-specific induced gene. *Plant Cell Physiol* 44(11):1208–1214
- Donaldson P, Anderson T, Lane B, Davidson A, Simmonds D (2001) Soybean plants expressing an active oligomeric oxalate oxidase from the wheat *gf-2.8* (germin) gene are resistant to the oxalate-secreting pathogen *sclerotinia sclerotiorum*. *Physiol Mol Plant Pathol* 59(6):297–307
- Druka A, Kudrna D, Kannangara CG, von Wettstein D, Kleinohs A (2002) Physical and genetic mapping of barley (*Hordeum vulgare*) germin-like cDNAs. *Proc Natl Acad Sci USA* 99(2):850–855
- Dumas B, Sailland A, Cheviet JP, Freyssinet G, Pallett K (1993) Identification of barley oxalate oxidase as a germin-like protein. *C R Acad Sci, 3 Sci Vie* 316(8):793–798
- Dunwell J, Gane P (1998) Microbial relatives of seed storage proteins: conservation of motifs in a functionally diverse superfamily of enzymes. *J Mol Biol* 46(2):147–154
- Dunwell J, Gibbins J, Mahmood T, Naqvi SS (2008) Germin and germin-like proteins: evolution, structure, and function. *Crit Rev Plant Sci* 27:342–375
- Dunwell JM (1998) Cupins: a new superfamily of functionally diverse proteins that include germins and plant storage proteins. *Biotechnol Genet Eng Rev* 15:1–32
- Dunwell JM, Khuri S, Gane PJ (2000) Microbial relatives of the seed storage proteins of higher plants: conservation of structure and diversification of function during evolution of the cupin superfamily. *Microbiol Mol Biol Rev* 64(1):153–179
- Dunwell JM, Purvis A, Khuri S (2004) Cupins: the most functionally diverse protein superfamily? *Phytochemistry* 65(1):7–17
- Eulgem T (2005) Regulation of the arabidopsis defense transcriptome. *Trends Plant Sci* 10(2):71–8

- Federico ML, Iñiguez-Luy FL, Skadsen RW, Kaeppler HF (2006) Spatial and temporal divergence of expression in duplicated barley germin-like protein-encoding genes. *Genetics* 174(1):179–190
- Felle HH, Herrmann A, Hüchelhoven R, Kogel K-H (2005) Root-to-shoot signalling: apoplastic alkalization, a general stress response and defence factor in barley (*hordeum vulgare*). *Protoplasma* 227(1):17–24
- Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. *Proc Natl Acad Sci USA* 100(25):15253–15258
- Ficke A, Gadoury DM, Seem RC (2002) Ontogenic resistance and plant disease management: a case study of grape powdery mildew. *Phytopathology* 92(6):671–675
- Finn RD, Tate J, Misty J, Coghill PC, Sammut SJ, Hotz H-R, Ceric G, Forslund K, Eddy SR, Sonnenhammer ELL, Bateman A (2008) The pfam protein families database. *Nucleic Acids Res* 36(Database issue):D281–D288
- Fu D, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen X, Sela H, Fahima T, Dubcovsky J (2009) A kinase-start gene confers temperature-dependent resistance to wheat stripe rust. *Science* 323:1357–1360
- Fukuoka S, Saka N, Koga H, Ono K, Shimizu T, Ebana K, Hayashi N, Takahashi A, Hirochika H, Okuno K, Yano M (2009) Loss of function of a proline-containing protein confers durable disease resistance in rice. *Science* 325(5943):998–1001
- Gallegos MT, Schleif R, Bairoch A, Hofmann K, Ramos JL (1997) Arac/xyls family of transcriptional regulators. *Microbiol Mol Biol Rev* 61(4):393–410
- Gane PJ, Dunwell JM, Warwicker J (1998) Modeling based on the structure of vicilins predicts a histidine cluster in the active site of oxalate oxidase. *J Mol Biol* 46(4):488–493
- Gill BS, Appels R, Botha-Oberholster A-M, Buell CR, Bennetzen JL, Chalhoub B, Chumley F, Dvorak J, Iwanaga M, Keller B, Li W, McCombie WR, Ogihara Y, Quetier F, Sasaki T (2004) A workshop report on wheat genome sequencing: International genome research on wheat consortium. *Genetics* 168(2):1087–1096
- Godfrey D, Able AJ, Dry IB (2007) Induction of a grapevine germin-like protein (*vgglp3*) gene is closely linked to the site of *Erysiphe necator* infection: a possible role in defense? *Mol Plant-Microb Interact* 20(9):1112–1125
- Heintzen C, Fischer R, Melzer S, Kappeler K, Apel K, Staiger D (1994) Circadian oscillations of a transcript encoding a germin-like protein that is associated with cell walls in young leaves of the long-day plant *sinapis alba* L. *Plant Physiol* 106(3):905–915
- Himmelbach A, Liu L, Zierold U, Altschmied L, Maucher H, Beier F, Müller D, Hensel G, Heise A, Schützendübel A, Kumlehn J, Schweizer P (2010) Promoters of the barley germin-like *ger4* gene cluster enable strong transgene expression in response to pathogen attack. *Plant Cell* 22:937–952
- Houde M, Diallo AO (2008) Identification of genes and pathways associated with aluminum stress and tolerance using transcriptome profiling of wheat near-isogenic lines. *BMC Genomics* 9:400
- Hu K-M, Qiu D-Y, Shen X-L, Li X-H, Wang S-P (2008) Isolation and manipulation of quantitative trait loci for disease resistance in rice using a candidate gene approach. *Mol Plant* 1(5):786–793
- Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. *Genetics* 164(2):655–664
- Hurkman W, Tao H, Tanaka C (1991) Germin-like polypeptides increase in barley roots during salt stress. *Plant Physiol* 97(1):366–374
- Hurkman WJ, Lane BG, Tanaka CK (1994) Nucleotide sequence of a transcript encoding a germin-like protein that is present in salt-stressed barley (*hordeum vulgare* L.) roots. *Plant Physiol* 104(2):803–804
- Initiative TIB, investigators P, Vogel JP, Garvin DF, Mockler TC, Schmutz J, Rokhsar D, Bevan MW, sequencing D, assembly Barry K, Lucas S, Harmon-Smith M, Lail K, Tice H, Leader JS, Grimwood J, McKenzie N, Bevan MW, assembly P, end sequencing B, Huo N, Gu YQ, Lazo GR, Anderson OD, Leader JPV, You FM, Luo M-C, Dvorak J, Wright J, Febrer M, Bevan MW, Idziak D, Hasterok R, Garvin DF, sequencing T, analysis Lindquist E, Wang M, Fox SE, Priest HD, Filichkin SA, Givan SA, Bryant DW, Chang JH, Leader TCM, Wu H, Wu W, Hsia A-P, Schnable PS, Kalyanaraman A, Barbazuk B, Michael TP, Hazen SP, Bragg JN, Laudencia-Chingcuanco D, Vogel JP, Garvin DF, Weng Y, McKenzie N, Bevan MW, analysis G, annotation Haberer G, Spannagl M, Leader KM, Rattei T, Mitros T, Rokhsar D, Lee S-J, Rose JKC, Mueller LA, York TL, analysis R, Leader TW, Buchmann JP, Tanskanen J, Leader AHS, Gundlach H, Wright J, Bevan M, de Oliveira AC, da C Maia L, Belknap W, Gu YQ, Jiang N, Lai J, Zhu L, Ma J, Sun C, Pritham E, Genomics C, Leader JS, Murat F, Abrouk M, Haberer G, Spannagl M, Mayer K, Bruggmann R, Messing J, You FM, Luo M-C, Dvorak J, analysis SR, Fahlgren N, Fox SE, Sullivan CM, Mockler TC, Carrington JC, Chapman EJ, May GD, Zhai J, Ganssmann M, Gurazada SGR, German M, Meyers BC, Leader PJG, annotation M, gene family analysis, Bragg JN, Tyler L, Wu J, Gu YQ, Lazo GR, Laudencia-Chingcuanco D, Thomson J, Leader JPV, Hazen SP, Chen S, Scheller HV, Harholt J, Ulvskov P, Fox SE, Filichkin SA, Fahlgren N, Kimbrel JA, Chang JH, Sullivan CM, Chapman EJ, Carrington JC, Mockler TC, Bartley LE, Cao P, Jung K-H, Sharma MK, Vega-Sanchez M, Ronald P, Dardick CD, Bodt SD, Verelst W, Inzé D, Heese M, Schnittger A, Yang X, Kalluri UC, Tuskan GA, Hua Z, Vierstra RD, Garvin DF, Cui Y, Ouyang S, Sun Q, Liu Z, Yilmaz A, Grotewold E, Sibout R, Hematy K, Mouille G, Höfte H, Michael T, Pelloux J, O'Connor D, Schnable J, Rowe S, Harmon F, Cass CL, Sedbrook JC, Byrne ME, Walsh S, Higgins J, Bevan M, Li P, Brutnell T, Unver T, Budak H, Belcram H, Charles M, Chalhoub B, Baxter I (2010) Genome sequencing and analysis of the model grass *brachypodium distachyon*. *Nature* 463(7282):763–768
- Jones JDG, Dangl JL (2006). The plant immune system. *Nature* 444(7117):323–329
- Kafri R, Levy M, Pilpel Y (2006) The regulatory utilization of genetic redundancy through responsive backup circuits. *Proc Natl Acad Sci USA* 103(31):11653–11658
- Ke Y, Han G, He H, Li J (2009) Differential regulation of proteins and phosphoproteins in rice under drought stress. *Biochem Biophys Res Commun* 379(1):133–138
- Khuri S, Bakker FT, Dunwell JM (2001) Phylogeny, function, and evolution of the cupins, a structurally conserved, functionally diverse superfamily of proteins. *Mol Biol Evol* 18(4):593–605
- Klink VP, Overall CC, Alkharouf NW, MacDonald MH, Matthews BF (2007) Laser capture microdissection (lcm) and comparative microarray expression analysis of syncytial cells isolated from incompatible and compatible soybean

- (glycine max) roots infected by the soybean cyst nematode (*Heterodera glycines*). *Planta* 226(6):1389–1409
- Knecht K, Seyffarth M, Desel C, Thureau T, Sherameti I, Lou B, Oelmüller R, Cai D (2010) Expression of *byglp-1* encoding a germin-like protein from sugar beet in *Arabidopsis thaliana* leads to resistance against phytopathogenic fungi. *Mol Plant-Microb Interact* 23(4):446–457
- Kondo K, Yamada K, Nakagawa A, Takahashi M, Morikawa H, Sakamoto A (2008) Molecular characterization of atmospheric NO₂-responsive germin-like proteins in *azalea* leaves. *Biochem Biophys Res Commun* 377(3):857–861
- Kou Y, Wang S (2010) Broad-spectrum and durability: understanding of quantitative disease resistance. *Curr Opin Plant Biol* 13(2):181–185
- Kouranov A, Xie L, de la Cruz J, Chen L, Westbrook J, Bourne PE, Berman HM (2006) The RCSB PDB information portal for structural genomics. *Nucleic Acids Res* 34(Database issue):D302–D305
- Krattinger S, Lagudah E, Spielmeier W, Singh R, Huerta-Espino J, McFadden H, Bossolini E, Selter L, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 323(5919):1360–1363
- Kukavica B, Vucinić Z, Vuletić M (2005) Superoxide dismutase, peroxidase, and germin-like protein activity in plasma membranes and apoplast of maize roots. *Protoplasma* 226(3–4):191–197
- Lai J, Li Y, Messing J, Dooner HK (2005) Gene movement by helitron transposons contributes to the haplotype variability of maize. *Proc Natl Acad Sci USA* 102(25):9068–9073
- Lane BG (1994) Oxalate, germin, and the extracellular matrix of higher plants. *FASEB J* 8(3):294–301
- Lane BG (2002) Oxalate, germins, and higher-plant pathogens. *IUBMB Life* 53(2):67–75
- Lane BG, Bernier F, Dratewka-Kos E, Shafai R, Kennedy TD, Pyne C, Munro JR, Vaughan T, Walters D, Altomare F (1991) Homologies between members of the germin gene family in hexaploid wheat and similarities between these wheat germins and certain physarum spherulins. *J Biol Chem* 266(16):10461–10469
- Lane BG, Cuming AC, Frégeau J, Carpita NC, Hurkman WJ, Bernier F, Dratewka-Kos E, Kennedy TD (1992) Germin isoforms are discrete temporal markers of wheat development. Pseudogermin is a uniquely thermostable water-soluble oligomeric protein in ungerminated embryos and like germin in germinated embryos, it is incorporated into cell walls. *Eur J Biochem* 209(3):961–969
- Lane BG, Dunwell JM, Ray JA, Schmitt MR, Cuming AC (1993) Germin, a protein marker of early plant development, is an oxalate oxidase. *J Biol Chem* 268(17):12239–12242
- Li Q, Li L, Dai J, Li J, Yan J (2009) Identification and characterization of cacta transposable elements capturing gene fragments in maize. *Chin Sci Bull* 42:251–269
- Liang H, Maynard CA, Allen RD, Powell WA (2001) Increased septoria musiva resistance in transgenic hybrid poplar leaves expressing a wheat oxalate oxidase gene. *Plant Mol Biol* 45(6):619–629
- Liu G, Jia Y, Correa-Victoria FJ, Prado GA, Yeater KM, McClung A, Correll JC (2009) Mapping quantitative trait loci responsible for resistance to sheath blight in rice. *Phytopathology* 99(9):1078–1084
- Lou Y, Baldwin IT (2006) Silencing of a germin-like gene in *Nicotiana attenuata* improves performance of native herbivores. *Plant Physiol* 140(3):1126–1136
- Mahmood T, Nazar N, Abbasi B (2010) Comparative analysis of regulatory elements in different germin-like protein gene promoters. *Afr J Biotechnol* 9(13):1871–1881
- Manosalva P, Davidson R, Liu B, Zhu X, Hulbert S, Leung H, Leach J (2008) A germin-like protein gene family functions as a complex QTL conferring broad-spectrum disease resistance in rice. *Plant Physiol* 149(1):286–296
- Mathieu M, Lelu-Walter MA, Blervacq AS, David H, Hawkins S, Neutelings G (2006) Germin-like genes are expressed during somatic embryogenesis and early development of conifers. *Plant Mol Biol* 61(4–5):615–627
- McDowell JM, Simon SA (2008) Molecular diversity at the plant-pathogen interface. *Dev Comp Immunol* 32(7):736–744
- Membré N, Bernier F, Staiger D, Berna A (2000) *Arabidopsis thaliana* germin-like proteins: common and specific features point to a variety of functions. *Planta* 211(3):345–354
- Membré N, Berna A, Neutelings G, David A, David H, Staiger D, Vázquez JS, Raynal M, Delseny M, Bernier F (1997) cDNA sequence, genomic organization and differential expression of three *Arabidopsis* genes for germin/oxalate oxidase-like proteins. *Plant Mol Biol* 35(4):459–469
- Mills EN, Jenkins J, Marigheto N, Belton PS, Gunning AP, Morris VJ (2002) Allergens of the cupin superfamily. *Biochem Soc Trans* 30(Pt 6):925–929
- Ohno S (1970) Evolution by gene duplication. Springer-Verlag, Berlin
- Ouyang S, Buell CR (2004) The tigr plant repeat databases: a collective resource for the identification of repetitive sequences in plants. *Nucleic Acids Res* 32(Database issue):D360–D363
- Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J, Buell CR (2007) The tigr rice genome annotation resource: improvements and new features. *Nucleic Acids Res* 35(Database issue):D883–D887
- Padmanabhan M, Cournoyer P, Dinesh-Kumar SP (2009) The leucine-rich repeat domain in plant innate immunity: a wealth of possibilities. *Cell Microbiol* 11(2):191–198
- Park C-J, Kim K-J, Shin R, Park JM, Shin Y-C, Paek K-H (2003) Pathogenesis-related protein 10 isolated from hot pepper functions as a ribonuclease in an antiviral pathway. *Plant J* 37(2):186–198
- Pinson SRM, Capdevielle FM, Oard JH (2005) Confirming QTLs and finding additional loci conditioning sheath blight resistance in rice using recombinant inbred lines. *Crop Sci* 45:313–324
- Ramalingam J, Cruz CMV, Kukreja K, Chittoor JM, Wu JL, Lee SW, Baraoidan M, George ML, Cohen MB, Hulbert SH, Leach JE, Leung H (2003) Candidate defense genes from rice, barley, and maize and their association with qualitative and quantitative resistance in rice. *Mol Plant-Microb Interact* 16(1):14–24
- Ramputh A, Arnason J, Cass L, Simmonds J (2002) Reduced herbivory of the European corn borer (*Ostrinia nubilalis*) on corn transformed with germin, a wheat oxalate oxidase gene. *Plant Sci* 162(3):431–440
- Requena L, Bornemann S (1999) Barley (*Hordeum vulgare*) oxalate oxidase is a manganese-containing enzyme. *Biochem J* 343 Pt 1:185–190
- Rodríguez-López M, Baroja-Fernández E, Zanduetta-Criado A, Moreno-Bruna B, Muñoz FJ, Akazawa T, Pozueta-Romero J (2001) Two isoforms of a nucleotide-sugar pyrophosphatase/phosphodiesterase from barley leaves (*Hordeum vulgare* L.) are distinct oligomers of HvGLP1, a germin-like protein. *FEBS Lett* 490(1–2):44–48
- Salse J, Chague V, Bolot S, Magdelenat G, Huneau C, Pont C, Belcram H, Couloux A, Gardais S, Evrard A, Segurens

- B, Charles M, Ravel C, Samain S, Charmet G, Boudet N, Chalhou B (2008) New insights into the origin of the b genome of hexaploid wheat: evolutionary relationships at the spa genomic region with the s genome of the diploid relative *aegilops speltoides*. *BMC Genomics* 9(1):555
- Schnable P, Ware D, Fulton R, Stein J, Wei F, Pasternak S, Liang C, Zhang J, Fulton L, Graves T, Minx P, Reily A, Courtney L, Kruchowski S, Tomlinson C, Strong C, Delehaunty K, Fronick C, Courtney B, Rock S, Belter E, Du F, Kim K, Abbott R, Cotton M, Levy A, Marchetto P, Ochoa K, Jackson S, Gillam B, Chen W, Yan L, Higginbotham J, Cardenas M, Waligorski J, Applebaum E, Phelps L, Falcone J, Kanchi K, Thane T, Scimone A, Thane N, Henke J, Wang T, Ruppert J, Shah N, Rotter K, Hodges J, Ingenthron E, Cordes M, Kohlberg S, Sgro J, Delgado B, Mead K, Chinwalla A, Leonard S, Crouse K, Collura K, Kudrna D, Currie J, He R, Angelova A, Rajasekar S, Mueller T, Lomeli R, Scara G, Ko A, Delaney K, Wissotski M, Lopez G, Campos D, Braidotti M, Ashley E, Golser W, Kim H, Lee S, Lin J, Dujmic Z, Kim W, Talag J, Zuccolo A, Fan C, Sebastian A, Kramer M, Spiegel L, Nascimento L, Zutavern T, Miller B, Ambroise C, Muller S, Spooner W, Narechania A, Ren L, Wei S, Kumari S, Faga B, Levy M, McMahan L, Buren PV, Vaughn M, Ying K, Yeh C-T, Emrich S, Jia Y, Kalyanaraman A, Hsia A-P, Barbazuk W, Baucom R, Brutnell T, Carpita N, Chaparro C, Chia J-M, Deragon J-M, Estill J, Fu Y, Jeddeloh J, Han Y, Lee H, Li P, Lisch D, Liu S, Liu Z, Nagel D, McCann M, SanMiguel P, Myers A, Nettleton D, Nguyen J, Penning B, Ponnala L, Schneider K, Schwartz D, Sharma A, Soderlund C, Springer N, Sun Q, Wang H, Waterman M, Westerman R, Wolfgruber T, Yang L, Yu Y, Zhang L, Zhou S, Zhu Q, Benetzen J, Dawe R, Jiang J, Jiang N, Presting G, Wessler S, Aluru S, Martienssen R, Clifton S, McCombie W, Wing R, Wilson R (2009) The b73 maize genome: complexity, diversity, and dynamics. *Science* 326(5956):1112–1115
- Schulte D, Close TJ, Graner A, Langridge P, Matsumoto T, Muehlbauer G, Sato K, Schulman AH, Waugh R, Wise RP, Stein N (2009) The international barley sequencing consortium—at the threshold of efficient access to the barley genome. *Plant Physiol* 149(1):142–147
- Schweizer P, Christoffel A, Dudler R (1999) Transient expression of members of the germin-like gene family in epidermal cells of wheat confers disease resistance. *Plant J* 20(5):541–552
- Sirithunya P, Tragoonrungs S, Vanavichit A, Pa-In N, Vongsaprom C, Toojinda T (2002) Quantitative trait loci associated with leaf and neck blast resistance in recombinant inbred line population of rice (*oryza sativa*). *DNA Res* 9(3):79–88
- Soares NC, Francisco R, Vielba JM, Ricardo CP, Jackson PA (2009) Associating wound-related changes in the apoplast proteome of medicago with early steps in the ros signal-transduction pathway. *J Proteome Res* 8(5):2298–309
- Soisson SM, MacDougall-Shackleton B, Schleif R, Wolberger C (1997a) The 1.6 Å crystal structure of the arac sugar-binding and dimerization domain complexed with d-fucose. *J Mol Biol* 273(1):226–237
- Soisson SM, MacDougall-Shackleton B, Schleif R, Wolberger C (1997b) Structural basis for ligand-regulated oligomerization of arac. *Science* 276(5311):421–425
- Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *xa21*. *Science* 270(5243):1804–1806
- Tabien E, Li Z, Paterson H, Marchetti A, Stansel W, Pinson M (2002) Mapping qtls for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. *Theor Appl Genet* 105(2–3):313–324
- Tanner A, Bornemann S (2000) *Bacillus subtilis* yvrk is an acid-induced oxalate decarboxylase. *J Bacteriol* 182(18):5271–5273
- Wei Y, Zhang Z, Andersen CH, Schmelzer E, Gregersen PL, Collinge DB, Smedegaard-Petersen V, Thordal-Christensen H (1998) An epidermis/papilla-specific oxalate oxidase-like protein in the defence response of barley attacked by the powdery mildew fungus. *Plant Mol Biol* 36(1):101–112
- Wisser RJ, Sun Q, Hulbert SH, Kresovich S, Nelson RJ (2005) Identification and characterization of regions of the rice genome associated with broad-spectrum, quantitative disease resistance. *Genetics* 169(4):2277–2293
- Wisser RJ, Balint-Kurti PJ, Nelson, RJ (2006) The genetic architecture of disease resistance in maize: a synthesis of published studies. *Phytopathology* 96(2):120–129
- Wojtaszek P (1997a) Mechanisms for the generation of reactive oxygen species in plant defence response. *Acta Physiol Plant* 19:581–589
- Wojtaszek P (1997b) Oxidative burst: an early plant response to pathogen infection. *Biochem J* 322(Pt 3):681–692
- Woo EJ, Dunwell JM, Goodenough PW, Marvier AC, Pickersgill RW (2000) Germin is a manganese containing homohexamer with oxalate oxidase and superoxide dismutase activities. *Nat Struct Biol* 7(11):1036–1040
- Wu J-L, Sinha PK, Variar M, Zheng K-L, Leach JE, Courtois B, Leung H (2004) Association between molecular markers and blast resistance in an advanced backcross population of rice. *Theor Appl Genet* 108(6):1024–1032
- Xiang P, Beardslee TA, Zeece MG, Markwell J, Sarath G (2002) Identification and analysis of a conserved immunoglobulin e-binding epitope in soybean *g1a* and *g2a* and peanut *ara h 3* glycinins. *Arch Biochem Biophys* 408(1):51–57
- Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *pm3b* from hexaploid wheat. *Plant J* 37(4):528–538
- Yamahara T, Shiono T, Suzuki T, Tanaka K, Takio S, Sato K, Yamazaki S, Satoh T (1999) Isolation of a germin-like protein with manganese superoxide dismutase activity from cells of a moss, *barbula unguiculata*. *J Biol Chem* 274(47):33274–33278
- Yi H, Richards EJ (2007) A cluster of disease resistance genes in arabidopsis is coordinately regulated by transcriptional activation and rna silencing. *Plant Cell* 19(9):2929–2939
- Zimmermann G, Bäumlein H, Mock H-P, Himmelbach A, Schweizer P (2006) The multigene family encoding germin-like proteins of barley. Regulation and function in basal host resistance. *Plant Physiol* 142(1):181–192
- Zou J, Pan X, Chen Z, Xu J, Lu J, Zhai W, Zhu L (2000) Mapping quantitative trait loci controlling sheath blight resistance in two rice cultivars (*oryza sativa* l.). *Theor Appl Genet* 101:569–573